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(57) Abstract

Methods and kits for measuring mutant hypersensitivity assay using high-throughput screening methodology to evaluate the mechanisms of toxicity of chemicals. The assay is performed in multi-well plates, such as those having 96 wells, making the process conducive to testing many compounds in a short period of time. The assay is versatile in that it can test compounds for ability to cause, for example, DNA damage, ability to mutate genetic material (mutagenicity), the ability to cause cancer (carcenogenicity), cause protein or membrane damage, energy depletion, mitochondrial damage, as well as the more general genotoxicity. Thus, the term toxicity, as used in this disclosure, is intended to encompass all of these types of effects. Furthermore, the assay can detect oxidative stress, protein damage, cell cycle disruption, energy charge and depletion, microtubule disruption or onset of metabolic competency through overexpression of human gene inserts encoding metabolism genes or incorporation of S9 fraction. In a preferred embodiment of the present invention, wildtype (wt) yeast and respective mutants are dosed with the desired chemical and yeast growth is determined using turbidimetry. Dose response curves are generated and mutant sensitivity to the compound relative to its parent (relative sensitivity) calculated. Relative sensitivities which are statistically significant indicate a hypersensitivity of the mutant to the test compound. The assay therefore provides an inexpensive, reliable, short term toxicity test which is an excellent alternative to animal testing and which provides valuable information about the mechanism of action of a compound. The present invention has applications to the pharmaceutical industry, environmental testing and clinical studies.

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HIGH-THROUGHPUT TOXICOLOGICAL TESTING USING CULTURED ORGANISMS AND CELLS

Field of the Invention

The present invention provides methods and diagnostic kits for identifying and characterizing toxic compounds. These methods and diagnostic kits measure reduced growth, as revealed by reduced relative viability of mutant as compared to wildtype, in samples of cultured organisms or cells exposed to the test compounds.

Background of the Invention

Many situations call for a way to determine whether a chemical has a toxic effect on living things. Such testing could be for a newly developed chemical or drug, as mandated by the Environmental Protection Agency ("EPA") or Food and Drug Administration ("FDA"). Alternatively, an environmental sample could be suspected of containing a toxin, and such testing could establish its presence. Because the investigator seeks the effect of the sample on biological entities, testing methods have focused on bioassay procedures. A wide variety of living things have been used in such assays, ranging from single cells to multicellular, complex organisms such as plants and animals. The large majority of toxicity testing, particularly that required by the government, involves toxicity tests in animals.

Toxicity testing in animals is expensive, time consuming, is relatively inaccurate, and can result in animal suffering. A two year toxicity test performed in rats was estimated to cost $800,000 in 1991. Louis J. Casarett et al., Casarett and Doull's Toxicology 37 (4th ed. 1991). Although the time required for animal toxicity tests varies with the species, short term tests run 3 months to 2 years and long term tests last two to seven years. Even after spending that amount of money and

investing that amount of time, the large number of variables present in animal testing, such as their diet, has been shown to effect the accuracy of the findings. Lastly, animal rights activists have made the general public aware of the issue of animal suffering during these tests, causing companies to seek out alternative testing methods. In short, animal testing has proven to be a very inefficient method of determining whether a sample is toxic, and there is an urgent need for a quick, inexpensive and reliable means of determining toxicity.

The prior art describes several different types of short term bioassays, usually detecting mutagenicity or carcinogenicity. The Ames assay and U.S. Patent No. 4,256,832, issued to Findl et al. are two examples of assays which detect reversion of a mutant organism to wildtype. As such reversion is generally the result of DNA mutation, positive results indicate an effect at the DNA level. U.S. Patent No. 4,997,757, issued to Schiestl, describes a yeast bioassay which measures the ability of the sample to induce genome arrangement. Again, this bioassay looks only at effects at the DNA level and may well miss non-mutagenic or non-carcinogenic toxins. To avoid this limitation, other functions of single cells have been used as the basis for toxicity assays. Farr teaches toxicity measurements made at a transcription or translation level in U.S. Patent Nos. 5,585,232 and 5,589,337. Botsford, in U.S. Patent No. 5,792,622, describes an assay which quantitatively measures inhibition of the electron transport chain by a test substance, and Hirth, in U.S. Patent No. 5,763,198, describes an assay which measure the tyrosine phosphorylation state of a protein substrate. However, all of these tests suffer from relatively complex genetic preparation of the organisms to be used or measures characteristics which may not be affected by a wide range of toxins. Accordingly, there remains a need in the art for an inexpensive, reliable bioassay with low start-up costs which can rapidly detect toxicity in compounds

having a wide range of effects, as well as provide mechanistic information about the subcellular targets of toxicity

Summary of the Invention

The present invention are methods and kits for measuring mutant hypersensitivity assay using high throughput screening methodology to evaluate the mechanisms of toxicity of chemicals. The assay is performed in multi-well plates, such as those having 96 wells, making the process conducive to testing many compounds in a short period of time. The assay is versatile in that it can test compounds for ability to cause, for example, DNA damage, ability to mutate genetic material (mutagenicity), the ability to cause cancer (carcenogenicity), cause protein or membrane damage, energy depletion, mitochondrial damage, as well as the more general genotoxicity. Thus, the term toxicity, as used in this disclosure, is intended to encompass all of these types of effects. Furthermore, the assay can detect oxidative stress, protein damage, cell cycle disruption, energy charge and depletion, microtubule disruption or onset of metabolic competency through overexpression of human gene inserts encoding metabolism genes or incorporation of S9 fraction. In a preferred embodiment of the present invention, wildtype (wt) yeast and respective mutants are dosed with the desired chemical and yeast growth is determined using turbidimetry. Dose response curves are generated and mutant sensitivity to the compound relative to its parent (relative sensitivity) calculated. Relative sensitivities which are statistically significant indicate a hypersensitivity of the mutant to the test compound. The assay therefore provides an inexpensive, reliable, short term toxicity test which is an excellent alternative to animal testing and which provides valuable information about the mechanism of

action of a compound. The present invention has applications to the pharmaceutical industry, environmental testing and clinical studies.

Brief Description of the Drawings

Fig. 1A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of acetylsalicyclic acid (aspirin). Fig. 1B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 1A. None of the sensitivity data fields are shaded, indicating all results are statistically insignificant.

Fig. 2A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of cimetidine (Tagamet®). Fig. 2B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 2A. None of the sensitivity data fields are shaded, indicating all results are statistically insignificant.

Fig. 3A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of oligomycin (antibiotic). Fig. 3B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 3A. None of the sensitivity data fields are shaded, indicating all results are statistically insignificant.

Fig. 4A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of actinomycin D (a DNA binder). Fig. 4B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of

Fig. 4A. The shaded data fields in Fig. 4B indicate statistically significant relative sensitivities.

Fig. 5A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of carboplatin (an antineoplastic alkylating agent). Fig. 5B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 5A. The shaded data fields in Fig. 5B indicate statistically significant relative sensitivities.

Fig. 6A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of EMS (ethyl methane sulfonate, an alkylating agent). Fig. 6B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 6A. The shaded data fields in Fig. 6B indicate statistically significant relative sensitivities.

Fig. 7A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of 5-FU (5-fluorouracil, an antimetabolite). Fig. 7B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 7A. The shaded data fields in Fig. 7B indicate statistically significant relative sensitivities.

Fig. 8A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of MMS (methyl methane sulfonate, an alkylating agent). Fig. 8B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 8A. The shaded data fields in Fig. 8B indicate statistically significant relative sensitivities.

Fig. 9A reports the undosed/dosed turbidity of wildtype (parental) yeast strains and mutant yeast strains in the presence varying concentrations of PMA (phorbol 12-acetate-13-myristate, a carcinogen). Fig. 9B shows the relative sensitivity of the wildtype (parental) yeast strains to the mutant yeast strains, as calculated from the data of Fig. 9A. The shaded data fields in Fig. 9B indicate statistically significant relative sensitivities.

Fig. 10 is a flow chart documenting a preferred embodiment of the assay of the present invention.

Detailed Description of Preferred Embodiments

The kits and methods of this invention utilize mutant organisms or cells where the mutation results in an alteration in at least one of multiple gene function categories. Organisms, which can be used in the present method, include any prokaryotic and eukaryotic unicellular organism such as the bacteria, E. coli, or the yeast, Saccharomyces cerevisiae. Use of cultured cell lines, particularly from mammalian species, is also contemplated for the present assay. The mutations present in the organisms or cell lines can be of any type, including lack of function or overexpression, as long as the ultimate phenotypic result is an unwanted adverse and hypersensitive effect which is toxicologically relevant.

Some gene function categories contemplated for the mutants for use in the kits and methods of the present invention include mutations which sensitize cells to, cause cells to exhibit, or disrupt a cell's function in acute phase stress, cell adhesion, AH-response, anti-apoptosis and apoptosis, antimetabolism, anti-proliferation, arachidonic acid release, ATP depletion, cell cycle disruption, cell matrix disruption, cell migration, cell proliferation, cell regeneration, cell-cell communication, cholestasis, differentiation, DNA damage, DNA replication, early

response genes, endoplasmic reticulum stress, estogenicity, fatty liver, fibrosis, general cell stress, glucose deprivation, growth arrest, heat shock, hepatotoxicity, hypercholesterolemia, hypoxia, immunotox, inflammation, invasion, ion transport, liver regeneration, cell migration, mitochondrial function, mitogenesis, multidrug resistance, nephrotoxicity, oxidative stress, peroxisome damage, recombination, ribotoxic or ribotoxic stress, sclerosis, steatosis, teratogenesis, transformation, disrupted translation, transport, or tumor suppression. In particular, the genes listed in Appendix A (yeast) and Appendix B (mammalian) are preferred for use in the present assay. Mutations affecting each of these gene function categories have been isolated and the gene or gene-associated sequence has been isolated and sequenced. Many mutants are available commercially, or can be easily constructed using standard genetic methods well known to one of ordinary skill, such as those described in Molecular Cloning — a Laboratory Manual, J. Sambrook et al., eds. (2d ed. 1989) and/or Molecular Biomethods Handbook, Ralph Rapley and John Walker, eds. (1998). Furthermore, homologous genes across species have been discovered through sequence homology, allowing similar testing to be done using mutations in bacteria, yeast, and mammalian cell lines. Thus, obtaining mutants for use in the assay of the present invention at any species level -- bacterial, yeast or mammalian -- is well within the purview of one of ordinary skill in this art.

Although many samples to be tested for cytotoxicity can be added directly to cells, the true form of the agent in the body is sometimes better represented if the sample if pre-treated with S9 fraction obtained from liver, or microsomes obtained from endoplasmic reticulum. See generally EM Gillam, Human cytochrome P450 enzymes expressed in bacteria: reagents to probe molecular interactions in toxicology, 25 Clin. Exp. Pharmacol. Physiol., 877(1998) and Paul S. Billings, et al., S-9 Metabolic Activation Enhances Aflatoxin-Mediated Transformation of

C3H/10T1/2 Cells, 77 Tox. Appl. Pharm.58 (1985); Robin E. Pearce, et al., Effects of Freezing, Thawing, and Storing Human Liver Microsomes on Cytochrome P450 Activity, 331 Arch. Biochem. Biophy. 145 (1996). Cytochrome P450s present in the fraction or microsome metabolize the foreign compound and sometimes activate it into a carcinogenic or toxic species. Thus, the present assay can be preceded with a metabolic activation step to insure the appropriate form of the sample is being tested. The S9 fraction and microsomes are available commercially from Xenotech (Kansas City, MS) and In vitro Technologies (IVT, Baltimore, MD). Incubation of the fraction or microsomes with the sample is done according to the protocol included with the commercial product. This process allows the sample to be in the form it would be in after metabolism in the organism, thus it is in the form that needs to be tested for an effect on the organism. If the metabolic activation step is used, only the resulting metabolites are actually tested in the assay, with non-treated sample included as a control. Pre-treatment with microsomes from various sources, such as human liver or yeast is contemplated.

Reduced growth by the mutant strain when dosed by the test sample indicates a potential toxic effect as well as the mechanism of action of the sample on living organisms. The method of measuring the growth of the organism depends on the particular one used. Bacteria and yeast growth can be followed using media turbidity measurements. Growth of cultured cell lines can be measured using radiolabelled compounds such as carbon 14 or 3H-thymidine incorporation or analysis using fluorescent dyes or colometric methods. Other possible ways of measuring cell culture growth are the MTT or XTT assay (measuring the reduction of a tetrazolium salt, such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenoltertazolum bromide, to a formazan product during growth), calcein AM, Trypan blue, neutral red uptake or colony formation method. These and other growth measuring

processes are well known to one of ordinary skill. The reduced growth data can be analyzed, finding IC 50 values, plotting dose response curves, and calculating relative and differential sensitivity of the mutant as compared to wildtype. Furthermore, testing using multiple mutants in different gene function categories can provide insights as to the subcellular target(s) of the tested substance when results vary by the gene function category of the mutant. Thus, not only can the assay be used to classify a particular sample as having a toxic effect, it may also be possible to pinpoint which cellular function or functions are being affected by exposure to the sample.

The first step of the present assay involves the selection of the mutant to be used in the assay. This decision is made based on the type of toxicity which is to be tested. For example, if a substance is to be tested for mutagenicity, then a mutation in a gene or genes encoding DNA damage or repair would be the most likely candidate. Thus, if no hypersensitivity to the substance is seen by the mutant through a reduced growth rate, it is likely that the substance does not have a mutagenic effect. If hypersensitivity of multiple DNA-related mutants is seen, it is likely that the substance does have a mutagenic effect, particularly if the hypersensitivity is not seen in mutations of other gene function catagories.

Yeast is an ideal organism for the present assay system because of its ease of cultivation, ease of generating gene knock-out mutants and extensive publicly available information about mutants. In particular, a yeast mutation hypersensitivity ("YMH") assay of the present invention could utilize one or more of literally hundreds of yeast mutations. Gene function categories of particular interest include, for example, cell rescue, defense, cell death and aging, cell growth, cell division, and DNA synthesis, heat shock proteins, mitochondrial, peroxisomal, DNA-associated, immunosuppressent, cyclins and cell cycle control proteins, ATP-

binding cassette proteins, cytoskeletal, metabolism and apoptosis. Appendix A of the present specification lists particular genes of interest in these and a "other relevant mutants" category. Presently, four mutant strains (Rad 6, Rad 18, Rad 51, and Rad 52) and two wild type strains of Saccharomyces cerevisiae (the parent strains of the mutations) have been tested against more than sixty compounds which range in toxicity from the alkylating agent methyl methane sulfonate (MMS) to acetominophenol. Each mutant has been selected based on its relevance to DNA damage repair mechanisms, a type of DNA-associated gene function. When a particular mutant is observed to be hypersensitive to a compound, it is indicative of chemical induced genotoxicity.

Rad 6 mutants are defective in repair of damaged DNA, mutagenesis and sporulation. The Rad 6 gene encodes a ubiquitin conjugating enzyme (E2) that is necessary for post-replication repair of damaged DNA and sporulation. Rad 6 exhibits an increase in transcription in response to DNA damage and during meiosis. Rad 6 encodes a protein of 172 amino acids which is a homologue of the mammalian ubiquitin carrier protein. Rad 6 mRNA levels have been shown to increase during sporulation and after exposure to UV light. However, no effect on transcription was observed after heat shock or starvation. These results suggest that Rad 6 is involved in genetic recombination repair rather than in the heat shock or stress response. If a mammalian cell line is preferred for the present method, a mammalian homologue of the Rad 6 gene is known -- HHR6B – and cells lines mutant in this gene are available from commercial sources, such as the American Tissue Culture Collection (ATCC, Bethesda, Md.)

The Rad 18 gene encodes a 66 kDa zinc finger protein that contains a nucleotide binding motif and the protein is required for repair of DNA damage and mutagenesis. Like Rad 6, Rad 18 is also involved in post-replication repair and

similarly, an increase in transcription is observed as a response to DNA damages. Rad 6 has been shown to form a heterodimeric complex with Rad 18 that has ubiquitin conjugating activity. The Rad 6-Rad 18 complex has been demonstrated to contain ATP hydrolytic activity in addition to binding single stranded DNA (ssDNA). Furthermore, Rad 6 alone has no binding affinity towards ssDNA but it is not essential that Rad 18 form a complex with Rad 6 in order for Rad 18 to bind ss DNA. Rad 18 might possibly target damaged sites on the DNA followed by Rad 6-directed ubiquitin mediated proteolysis.

Rad 51 is a structural and functional homologue of the bacterial strand exchange protein RecA. The Rad 51 gene product forms a complex with replication protein A (RPA) and ssDNA, and the protein is required for recombination and for x-ray damage repair. In addition, double stranded break (DSB) repair is dependent upon the Rad 51 gene. A mammalian equivalent of this gene is also known, HHR51.

The Rad 52 gene is necessary for mitotic recombination and repair of x-ray damage and DNA DSB. Rad 52 appears to bind Rad 51 to form a heterodimeric complex, which has an increased affinity toward ssDNA. Rad 52 protein enhances DNA strand exchange by causing Rad 51 protein to form a complex with RPA and ss DNA. As previously stated, formation of the Rad 51-RPA-ssDNA complex is an important intermediate in the recombination mechanism. A mammalian equivalent of this gene has been identified – HHR52.

Positive test compounds were selected by category of DNA damage. Some compounds tested thus far include antimetabolites such as hydroxyurea, methotrexate, and 5-fluorouracil. Alkylating agents such as cyclophosphamide, mitomycin C and ethyl methane sulfonate have also been tested. Also, examples of topoisomerase inhibitors, which include etopodide and camptothecin have been

tested. Other compounds such as the DNA binding compound Actinomycin D have been use in the assay. In addition innocuous compounds which should show no DNA damage effects are screened as negative controls. Well characterized compounds provide a test set of chemicals that can be compared against unknowns.

Control sets are introduced in the assay for verification and validation. Multiple wild type yeast from different parental strains are tested as an internal control. Since the Rad 6 and Rad 18 mutants are similar mutations in terms of DNA damage and repair mechanisms, comparison of the mutant hypersensitivity data of mutant strains can be used as a guide for experimental validation and interpretation of data. However, exclusive sensitivity of a single mutant has been observed. Therefore, strain comparison is only one aspect of the YMH assay used to verify the data. Other controls include undosed yeast, solvent controls and blanks. Also, experimental validation is performed using statistical analysis of the data, examination of standard deviations and coefficients of variation.

Once the assay is performed the data can be analyzed using several methods. One method looks at the IC 50 values for the parent and respective mutant strains. However, the IC 50 value may not be the best measure of mutant hypersensitivity especially when greater sensitivity is observed at concentrations other than IC 50. Mutant hypersensitivity can be observed throughout the dose response curve or conversely, mutant hypersensitivity might be seen in a narrow range of dosing concentrations. Therefore, plots of differential sensitivity (proliferation of mutant/proliferation of wt) or relative sensitivity (1-differential sensitivity) are constructed to evaluate the genotoxic effects of compounds.

With the assay of the present invention it may be possible to determine the mechanism of DNA damage that occurs which can be largely dependent upon the

mutant(s) that display hypersensitivity. For example, Rad 6 and Rad 18 may be hypersensitive to a compound whereas Rad 51 and Rad 52 may show no effect. That would indicate toxicity related to recombination repair as opposed to post-replication repair. Therefore, insight into the molecular mechanism of toxicity can be gained for target compounds.

Thus, the discussion above of the YMH assay can be summarized by the flow chart of Figure 10. First, the yeast strain of interest is selected where both the desired mutant and the parental strain is obtained. Cultures of both the wildtype and mutant are grown, and then dosed with the test substance. The dosed yeast, both mutant and wildtype are separately incubated in a 96 well plate. The plate is read for optical density and this data is used to detect any differences in growth in response to exposure to the test substance. The data is analyzed using various standard toxicity values, plotting methods, and comparisons. Finally, through particular selection of mutants and differential results in various gene function categories, the assay could provide information as to the mechanism of toxicity of the test substance.

A final embodiment of the present invention is a diagnostic kit which includes a battery of mutant organisms, and the parental strain for those mutants, where the mutants are selected to determine at what subcellular function the sample compound has its effect. An example of such a kit would include the Rad 6, Rad 18, Rad 51, and Rad 52 mutants and the parental strains. This kit would be useful in determining whether sample, suspected of having a DNA associated toxic effect, works through a double strand breaks or disruption of DNA replication.

Example 1

Use of Yeast for Cytotoxicity Assay

Mutant and respective wild type yeast are streaked on agar plates and incubated at 30° C. When isolated colonies formed (within 1 to 2 days), YM-1 stocks were prepared by innoculating a 15 ml sterile culture tubes containing 2.5 ml of YM-1 media. The tubes were incubated for about 18hrs in a shaking water bath set at 30° C and at least 137rpm. 2.5 ml of C media was placed in a 15 ml culture tube and this tube was innoculated with 100 µl of the YM-1 stocks prepared above. These tubes were incubated for about 4 hr. in a shaking water bath set at 30° C and at least 137 rpm. While these tubes were incubating, a dosing plate which contains the compound solution and dilutions was prepared.

It is preferable to prepare a dosing plate containing a serial dilution profile for various compounds that will be used to dose each yeast strain. This can be accomplished by constructing a masterplate that contains enough solution in each well such that multiple secondary plates can be produced by transferring 30 µl of each well in the master plate to corresponding wells in each of the secondary plates. When finished, all secondary plates will be identical, containing 30 µl per well of the desired compounds and concentrations. Stock dilutions should be made by solubilizing the compounds in water, DMSO, ethanol, or other appropriate liquid. DMSO or ethanol concentrations should not exceed about 0.1% including cells.

In a 96 well plate, use the stock solutions to make the desired dilutions that will be used to dose yeast. It is best to prepare dilutions in the 96 well plates using C media as diluent. Compounds solubilized in DMSO or ethanol should be diluted using C media containing 0.1% DMSO or ethanol. Similarly, DMSO and ethanol control wells should contain a final concentration of 0.1% of the appropriate solvent.

Up to 14 compounds can be tested at one time if the assay is done in a 96 well plate. A plate of this size can accommodate six different dosing concentrations with no replicates. In general, a maximum of seven compounds are screened against six yeast strains at six different compound concentrations performed in duplicate. The 96 well microplates that are utilized for the assay are usually prepared with a single yeast strain per plate. Conversely, one compound can be tested against multiple strains on each plate.

After about 4 hours, when the yeast solution in the 2.5 ml of C media is slightly turbid, 2.5 ml of yeast/C media culture prepared above should be combined with an additional 22.5 ml of C media, for a total volume of 25 ml. 270 µl of this diluted yeast solution prepared above is added to each well of a 96 well secondary dosing plate. Each diluted yeast strain (25ml) will be used for a single secondary dosing plate. Final volume in the well will be 300 µl. It is important that wild type and mutant strains have undosed control wells. A control well containing media and compound without yeast should also be prepared for background correction. The plates are incubated for 18-24 hr. at 30° C.

After removing the plates from the incubator they should be allowed to cool at room temperature for 15 min. Cooling may also be done at 4°C, as long as condensation is not formed during this process. Absorbence at 600 nm is read using a 96 well microplate reader. Readings can be performed at 0.1 seconds/well. Absorbence readings in the control wells should be between 0.3 and 1 absorbence units for reliable data.

The data is now ready for analysis. First, make any necessary background corrections to the data. Corrections can be made by subtracting the media blank absorbance from all absorbance values prior to generating dose response curves or calculating differential mutant sensitivity. From the spectophotometric data,

calculate the ratio of absorbance (dosed/undosed) for mutant and wild type strains at each compound dilution.

$$\text{Differential Sensitivity} = \frac{\text{Dosed/Undosed (mutant)}}{\text{Dosed/Undosed (wild type)}} \times 100$$

Relative Sensitivity = 1 - Differential Sensitivity

Or

$$\text{Relative Sensitivity} = \frac{\text{Dosed/Undosed (wild type)} - \text{Dosed/Undosed (mutant)}}{\text{Dosed/Undosed (wild type)}} \times 100$$

Note that Dosed/Undosed = Abs @ 600nm for dosed yeast/Abs @ 600 nm for undosed yeast.

The relative sensitivity is a measure of the effect of a compound on the mutant relative to the parent strain. Dose response curves can be compared between wild type and mutant strains.

Solutions Required

YM-1 Media	C media
In 250 ml of ddH_2O add:	In 250 ml of ddH_2O add:
1.25 g yeast extract	0.36 g yeast nitrogen base
2.5 g peptone	1.25 g ammonium sulfate
0.36 g yeast nitrogen base	1.5 g NaOH
1.25 g ammonium sulfate	5 g dextrose
1.5 g NaOH	0.35 g C powder

2.5 g succinic acid

0.01 g adenine

0.01 g uracil

5 g glucose

For this assay, the above formulas were used, but other variations well known to one of ordinary skill will be equally effective.

The results obtained indicate that the present assay succeeds in identifying compounds having toxic effects. Exposure to the three negative controls — aspirin, Tagamet®, and oligomycin — did not result in statistically significant relative sensitivity values, as determined by standard toxicological statistical processes. These results are recorded in Figures 1B, 2B, and 3B. In contrast, all the positive test substances resulted in statistically significant hypersensitivity on the part of the mutants. These results are recorded in Figures 4B, 5B, 6B, 7B, 8B, and 9B. Interestingly, actinomycin D, a DNA binder (Fig. 4B) resulted in hypersensitivity for Rad 6 and Rad 18 but not for Rad 51 or Rad 52. Rad 6 and Rad 18 are involved in post-replication repair. Rad 51 and Rad 52 are involved in doubled stranded break repair. From the data, this suggests that actinomycin D is a DNA damage agent that appears to damage DNA at the post-replication repair level but does not significantly alter the cells' ability to repair double strand breaks. Thus, actinomycin D can be classified as a mutagen that does not severely effect DNA replication. For example, this compound could bind the DNA such that replication occurs normally but mutations to the DNA are incorporated.

Example 2

Use of Mammalian Cell Cultures for Cytotoxicity Assay

A cell line having a desired mutation is selected for use in the assay. For example, the genes listed in Appendix B have been identified as appropriate for the present invention. Additionally, cell lines deficient in genes homologous to those preferred for the yeast assay, as listed in Appendix A, are also preferred for the mammalian embodiment of the present invention. The cell line is grown in appropriate media, such as DME + 10% calf serum (Dulbecco's Modification of Eagle's Basal Medium, Irvine Scientific, Santa Ana, CA), with appropriate characteristics for maintenance of the mutant's growth, if necessary. For effective growth measurements, the cells to be used must be made quiescent, that is, synchronized in growth stage at a low growth rate. This is done by seeding approximately 500,000 cells per well in a 24 well plate in the appropriate media. The cells are incubated for 12-24 hours at 37° C in a humidified incubator in an atmosphere of 5-7% CO_2, after which they are rinsed once in serum-free media. I ml of serum-free media is added to each well, and the cells are incubated for a further 24 hours. The sample to be tested is added to the wells in the appropriate concentrations. The cells are exposed to the sample for a 16 hour incubation. Then 1 micro curie of 3H-thymidine, 1µl diluted in 24 µl media, (New England Nuclear, #NET-027Z, Boston, MA) is added to each well. This mixture is allowed to incubate for 8 hours. The 3H-thymidine remaining unincorporated is extracted by aspirating the media, a careful wash with 1 ml ice cold PBS (phosphate buffered physiological saline solution), which is aspirated from the cells. ! ml of ice cold 5% trichloroacetic acid (TCA) is added to each well, and left at 4°C for 30 minutes. The TCA is aspirated off and the remaining cells are washed one time with PBS. At

room temperature, each well receives 0.5 ml of 0.5N NAOH/0.5% SDS. This is mixed by pipetting up and down, then placed in the scintillation vials for counting of the incorporated radioactivity. Similarly highthroughput screening could be done using CytostarT® plates (Amersham, Buckinghamshire, England), which avoids the TCA precipitation step. The higher the incorporation, the higher the growth rate of the cell line during exposure to the sample. The results can be interpreted as described above in Example 1 for the yeast cell assay.

Example 3

Use of Bacteria for Cytotoxicity Assay

A bacterial strain is selected that has a mutation in a gene function of interest. For example, genes having homologous functions as those listed as preferred for the yeast assay in Appendix A and the human cell assay listed in Appendix B are also preferred for the bacterial assay. Streak out the selected E. coli mutant and control parental strain on a solid agar plate, containing the appropriate selection agents to maintain the mutation. Incubate overnight at 37° C (or lower if mutant is temperature sensitive). Grow small cultures (5ml) from 5-10 isolated colonies in LB media (Luria-Bertani Medium). Incubate overnight at 37° C. Inoculate 5 ml of LB with 50 µl of each overnight culture. Incubate for 2 hours with aeration 2.5 ml of bacteria/LB culture prepared above should be combined with an additional 22.5 ml of LB media, for a total volume of 25 ml. 270 µl of this diluted bacteria solution prepared above is added to each well of a 96 well secondary dosing plate. Each diluted bacterial strain (25µl) will be used for a single secondary dosing plate. The secondary dosing plate is produced as discussed above for the yeast assay of Example 1. Final volume in the well will be 300 µl. It is important that parental and mutant strains have undosed control wells. A control well

containing media and compound without bacteria should also be prepared for background correction. The plates are incubated for 18-24 hr. at 37° C.

After removing the plates from the incubator they should be allowed to cool at room temperature for 15 min. Absorbance at 600 nm is read using a 96 well microplate reader. Readings can be performed at 0.1 seconds/well. The data is analyzed as described above in Example 1 for the yeast assay.

LB Media

To 950 ml of deionized H_2O, add:

Bacto-tryptone	10g
Bacto-yeast extract	5g
NaCl	10g

Shake until the solutes have dissolved. Adjust the pH to 7.0 with 5 N NaOH (about 0.2 ml). Adjust the volume of the solution to 1 liter with deionized H_2O. Sterilized by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle.

It is understood that the invention is not limited to the specific embodiments shown and described, but changes or modifications can be made in the embodiments without departed from the contemplated scope of the present invention. All such changes are apparent to one of ordinary skill in the art and all such changes and modifications are intended to be within the scope of the following claims. All references or patents discussed in this specification are hereby incorporated by reference.

Appendix A

NONLIMITING LIST OF MUTANTS AND GENES

CELL RESCUE, DEFENSE, CELL DEATH AND AGING

PRE3, PRE1, PUP2, RPN12, RPT1, MAG1, OGG1, SED1, ATH1, SPE2, GRE3, TPS2, TPS1, ATR1, ATX1, SKI3, SKI2, SKI8, APN1, HPR5, ERG5, CCZ1, SRA1, SNF1, YCK1, YCK2, HRR25, CTA1, CTT1, WSC4, PAM1, TIR2, TIR1, HDF2, TFB4, RAD1, HAM1, LYS7, SOD1, KIN28, DIT2, ERG11, CYC7, CCP1, PHR1, DAK2, DAK1, ALR1, ALR2, HOR2, RAD17, DDC1, DDR2, ALK1, HEL1, SSL2, RAD5, SGS1, PIF1, RAD3, CDC9, REV7, NTG1, RAD18, RAD57, RAD55, XRS2, RAD30, MMS21, RAD51, RAD10, PSO2, REV1, DIN7, RAD54, CDC2, PES4, POL2, REV3, RPB7, RPB4, SGE1, UBA1, UBC4, UBC5, RAD6, QRI8, RNC1, NTG2, ERC1, RAD4, ETH1, FKB2, YHB1, FLR1, MEC3, ZWF1, GSH1, GRX1, TTR1, HYR1, GLR1, YCF1, FPS1, GPD1, RAS2, RAS1, CUP5, HSP26, HSP30, HSP12, HSP104, DDR48, HSC82, HSP82, MDJ1, MDJ2, HSP60, HSP78, ECM10, SSE1, SSA1, SSA3, SSA4, SSA2, SSE2, HSF1, HIG1, HDF1, HMS2, GRE1, DDI1, RTA1, SIM1, LAG2, ZDS1, MET18, SNG1, NCA3, KTI12, UTH1, SUN4, SSU81, SSD1, THI4, KAR3, LIF1, SFA1, LAG1, LTV1, MDR1, SSK22, SSK2, HOL1, CIS3, HSP150, PIR3, MAC1, CUP1A, CUP1B, YDJ1, SSQ1, SSC1, IMP2, MPT5, ATX2, SNQ2, MLP1, NHX1, NCP1, NSR1, SNF4, RAD16, RAD7, RAD14, RAD23, ROD1, MGT1, OSM1, SIP18, SAT2, MNR2, MMS2, PNT1, CYP2, PAD1, PDR5, PDR3, PDR6, RTS1, PAI3, HOR7, DUN1, IRE1, MKK2, MET22, PPZ2, PTC1, PTP2, MMS4, RAD52, PDR13 SLG1, GRR1 HIT1, RDH54 BRO1, PIR1 MSRA, RNR4 RNR3, HAL1, YGP1, CDC55, PPZ1, PKC1, HAL5, MKK1, HOG1, SLT2, BCK1, RAD53, SIR4, SIR3, SIR2, MGA1, FUN30, YRO2, DNL4, RRD1, SAT4, RAD27, MSN2, STI1, PAU3, PAU2, PAU5, PAU1, PAU4, PAU6, (MLP1), RAD2, FZF1, SSU1, SOD2, CRS5, BCK2, ASM4, TIP1, TFB1, CCL1, SSL1, TFB3, TFB2, TSA1, TRX1, TRX2, ROX3, PDR1, GTS1, MCM1, SKN7, CAD1, MSN4, YAP1, SLN1, SSK1, PBS2, UBI4, RSP5, SVS1, ZRC1

CELL GROWTH, CELL DIVISION AND DNA SYNTHESIS

GSC2, PLC1, PRE3, PRE2, PRE1, PUP2, RPN12, RPT6, RPT1, DIS3, RP SOA,
AGA1, AGA2, ASG7, ACH1, ACT1, SAC6, ARP100, ABP1, PAN1, ARP 2, ARE1ARE2, SPE2, CYR1, SRV2, ADK2, GCS1, SOH1, TUB1, TUB3, SAG1, AKR1,

YAR1, SKI8, ARG82, ABF1, STE6, BAR1, BOI1, TUB2, RBL2, BIG1, BI M1,
BAT1, BEM1, BEM4, SBE2, BNI4, BUD6, BOI2, BUD9, BUD4, BUD8, RC K2,
CMK1, CNA1, CMP2, CNB1, CCH1, CMD1, SRA1, YCK1, YCK2, HRR2 5,
CKA2, CKA1, YCK3, EST2, TFS1, SCM4, GIC2, GIC1, CAK1, BUB2, B UB3,
ESR1, RAD24, DBF20, PDS1, HPC2, NUD1, CDC47, CDC10, CDC13, C DC37, CDC1, CDC40, CDC4, CDC20, CDC6, CDC46, CDC3, KAR1, BB P1, CDC50,
FUS1, KRE9, EGT2, ARP1, CHS1, CHS2, CHS3, CHS5, MSI1, CAC2, R LF2,
CHL4, SMC1, SMC2, CIN1, SNF7, CLC1, COF1, PAM1, LAS17, HDF2, SEC3,
SNF2, SWI1, SNF5, SNF11, DOC1, APC2, APC5, TAP42, CDC53, KAR 9,
CCE1, CLB6, CLB5, CLN3, PCL2, CLN1, PCL1, CLN2, CLB3, CLB1, CLB 4,
CLB2, FAR1, CKS1, CDC28, PHO85, KIN28, SSN3, CLG1, DIT2, SLA1, SLA2, SPO20, DPP1, RAD17, DDC1, HEL1, DNA2, RAD5, SGS1, HCS 1, PIF1, CDC9, MSH3, MSH6, MLH1, PMS1, MSH2, MSH1, POL4, REV 7, MRE11, RAD26,
RAD9, RAD18, RAD57, RAD55, XRS2, MMS21, RAD51, RAD10, RAD 50,
RFA3, RFA2, RFA1, RFC4, RFC5, RFC3, RFC2, RFC1, FOB1, TOP1, TO P2,
TOP3, RAP1, RAD54, PRI2, PRI1, POL1, POL12, CTF4, HUS2, CDC2, P ES4,
POL2, DPB2, DPB3, MIP1, REV3, SSN8, GAL11, RGR1, SRB6, RPO41, SEC59, DIP2, CDC14, MSG5, DYN1, UBC4, UBC9, CDC34, UBC5, UBC 1, UBC6,
RAD6, QRI8, ELC1, RNC1, CTS1, KEX2, APG1, SSP1, SUP35, EXM2, S PR1, EXG1, EXG2, DHS1, CAP1, CAP2, BRN1, GPR1, GIF1, MEC3, TU B4, CIS2,
LTE1, SDC25, SRM1, CDC25, ROM2, BUD5, ROM1, SPT16, CDC43, G IP1,
SIN4, SNF6, KRE6, GFA1, NGR1, WHI2, RSR1, CIN4, RAS2, RAS1, GP A1,
STE4, STE18, CDC42, MDG1, SEC4, TEM1, RHO3, RHO4, RHO2, RHO 1,
CDC24, BEM2, BUD2, BEM3, LRG1, GPA2, SIS1, HSP82, HSF1, ABF2, HDF1, HDR1, RPD3, HSL7, HO, SBA1, HPR1, IDS2, NFI1, CSE2, MDM 1, MUB1,

MID1, SIM1, HIR3, SIS2, MAK11, LAS1, SPA2, WHI4, ECM33, SET1, CTF19, CIN2, MCM16, SLK19, CYK2, CNM67, SST2, DPB11, DOS2, DFG16, AFR1,
ZDS1, SRO7, PEA2, FAR3, SMP2, WHI3, CDC5, MET30, SAS2, SCC2, CIS1,
STN1, UTH1, PAC2, SSD1, SRP1, KRE5, KIP1, CIN8, SMY1, KIP2, KAR3, KIP3, CBF1, CBF2, SKP1, CEP3, CTF13, DBR1, LAG1, MIH1, BFR1, DIG2, DIG1,
MFA1, MFA2, MFalpha1, MFAlpha2, MID2, SSF1, MATALPHA2, MATALPHA1, ALPHA1, ALPHA2, A2, A1, SAN1, PGD1, SPO11, MSH5, DMC1, ISC10,
MSH4, SPO13, NDT80, REC104, HOP1, RED1, SPO7, MUM2, MEI5, SAE2,
NAM8, REC107, REC102, REC114, MER1, RIM101, NDJ1, CDC54, CPR7,
SYG1, MCM2, CIS3, HSP150, ACE2, CDC48, ASE1, YTM1, HSM3, YDJ1,
ERV1, FUS3, JNM1, MCD1, MMC1, MSB1, MSB2, MPT5, ZDS2, MSN5, KEM1, MLC1, MYO2, MYO4, MYO5, MYO3, MYO1, DEC1, PMD1, MDS3, ASH1,
UME1, UME6, NHP6A, RFT1, TRF5, NNF1, NDC1, BIK1, KAR2, KAR5, NUM1, CDC39, MAK16, NAP1, RAD16, RAD23, NBP35, ORC1, ORC6, ORC5, ORC4, ORC3, RRR1, SIC1, BUD3, PWP2, STE3, STE2, OPY2, STE50, STE5, PEL1,
TOR1, TOR2, PIK1, STT4, MSS4, SPO14, POL32, IME4, SHP1, PDS5, FEN1,
CSE1, FLO8, PFY1, PHB2, PHB1, POL30, AXL1, STE23, RAD28, CDC7, SMP3, MKK2, CDC15, ARD1, CHL1, PPH3, PPH21, PPH22, PTC1, SEC9, PPS1, PTP3, YVH1, PTP2, PUS4, PCH2, PCH1, CBF5, SEF1, MMS4, SHR5, RAD59,
RAD52, RHC18, RGP1, RVS167, RIM9, BNR1, BNI1, SPT3, SOK2, KAR4,
DBF4, SDS22, MCM3, CTF18, SRO4, SPH1, FUS2, MOB1, FLO8, FIG1, FIG2,
END3, DFG5, CTR9, TOM1, POP2, GRR1, SCP160, SUR1, MUM3, ZIP2,
CDC45, RDH54, SHE3, SHE2, SHE4, GPI1, MIF2, ESP1, HOP2, DNA43, SMC3, PAC11, PAC10, RDI1, RGA1, RNR1, RNR2, RNR4, RNR3, PRPS1, RPL10,
RPS1A, MTF1, SNI2, CDC12, CDC11, SPR28, CDC55, GLC7, PKC1, GIN4,
SPS1, RCK1, BUB1, IME2, YAK1, YPK2, RIM11, CLA4, MKK1, MEK1, IPL1,

SGV1, SLT2, KSS1, BCK1, STE11, STE20, DBF2, HSL1, NRK1, SIT4, TPD3,
ELM1, MCK1, RAD53, STE7, SWE1, MPS1, SAS3, HST1, SIR4, SIR3, SIR1,
SIR2, CTH1, DOM34, HST4, RVS161, DNL4, IQG1, FUN16, HYM1, RTS2,
MNN10, PRK1, MCM6, SAP155, SAP4, SAP190, SAP185, MUD13, MAD1,
CIK1, NUF1, SPC97, SPC42, SPC98, CDC31, NUF2, MAD3, MAD2, DIT1,
YSW1, SPO12, SPO16, MCD4, BDF1, SGA1, GSG1, SHC1, CDA1, CDA2,
SMK1, SPS2, SPR6, SLZ1, SPS4, SPR3, SPS100, SPS18, RAD27, SNZ1,
SUR4, STI1, SBE22, CSE4, BMH1, SVL3, SCH9, (MLP1), SSF2, RAD2,
CDH1, CDC27, CDC26, CDC23, CDC16, APC1, APC11, APC4, APC9,
SAP30, RSC6, RSC8, STH1, SFH1, SAS5, JSN1, BMH2, SMT4, BCK2,
HOC1, ZIP1, UFE1,
EST1, TEL1, ANC1, CCL1, DST1, TRX1, TRX2, TRF4, PAT1, SPT4, SPT6,
CDC36, SWI5, SWI4, PHD1, SWI6, GTS1, MCM1, IME1, SKN7, MBP1,
SWI3, SIN3, STE12, CIN5, SDS3, SPO1, MOT2, RPG1, PRT1, CDC33,
TPM1, TPM2, TWF1, TEC1, TTP1, STE13, PRP8, UBI4, DSK2, RSP5, DOA4, UNG1, VPS45, VAN1, VRP1, DFG10, YHM2, GLO3, SFP1, STE24, RME1, SAE3, MEI4, NHP6B, MOB2, EST3, RIM1

HEAT SHOCK PROTEINS

CAT5, CPH1, CTT1, CYP2, DDR2, FPR2, HSC82, HSP104, HSP12, HSP150,
HSP26, HSP30, HSP42, HSP60, HSP78, HSP82, KAR2, MDJ1, SIS1, SOD2,
SSA1, SSA2, SSA3, SSA4, SSB1, SSB2, SSC1, SSE1, SSE2, STI1, TIP1,
TPS2, UBI4, YDJ1

MITOCHONDRIAL

AAC1, AAC3, AAT1, ABC1, ABF2, ACO1, ACR1, ADH3, ADK2, AEP2,
AFG3, ALD1, ALD2, ARG11, ARG2, ARG5,6, ARG7, ARG8, ARH1, ATM1, ATP1,
ATP10, ATP11, ATP12, ATP14, ATP15, ATP16, ATP2, ATP3, ATP4, A

TP5,
ATP6, ATP7, ATP8, ATP9, BAT1, BCS1, CBP1, CBP2, CBP3, CBP4, CB
P6,
CBR1, CBS1, CBS2, CCA1, CCE1, CCP1, CEM1, CIT1, CIT3, COB, CO
Q1,
COQ2, COQ3, COQ6, COR1, COT1, COX1, COX10, COX11, COX12, C
OX13, COX14, COX15, COX17, COX2, COX3, COX4, COX5A, COX5B
, COX6, COX7, COX8, COX9, CPR3, CTP1, CYB2, CYC1, CYC2, CYC3
, CYC7, CYT1, CYT2, DBI56, DLD1, DTP, ENS2, ERV1, FLX1, FUM1,
GCV1, GCV3, GLO4, GPD2,
GSD2, GUT2, HEM1, HEM15, HSP10, HSP60, HSP78, HTS1, IDH1, ID
H2,
IDP1, IFM1, ILV1, ILV2, ILV3, ILV5, ILV6, IMP1, IMP2, INH1, ISM1, KG
D1,
KGD2, LAT1, LEU4, LIP5, LPD1, LYS12, LYS4, MAE1, MAM33, MAS1,
MAS2, MBA1, MCR1, MDH1, MDJ1, MDJ2, MDM10, MDM12, MEF1,
MEF2, MET13, MGE1, MGM101, MIP1, MIR1, MIS1, MMM1, MMT1, M
MT2, MOD5, MOL1,
MRF1, MRP1, MRP13, MRP17, MRP2, MRP20, MRP21, MRP4, MRP49,
MRP51, MRP8, MRPL10, MRPL11, MRPL13, MRPL15, MRPL16, MRPL
17, MRPL19,
MRPL2, MRPL20, MRPL23, MRPL24, MRPL25, MRPL27, MRPL28, MRP
L3,
MRPL31, MRPL32, MRPL33, MRPL35, MRPL36, MRPL37, MRPL38, MR
PL39, MRPL4, MRPL40, MRPL44, MRPL49, MRPL6, MRPL7, MRPL8, M
RPL9, MRPS28, MRPS5, MRPS9, MRS1, MRS11, MRS2, MRS3, MRS4,
MRS5, MSD1, MSE1, MSF1, MSH1, MSK1, MSM1, MSP1, MSR1, MS
S1, MSS116, MSS18, MSS51, MST1, MSU1, MSW1, MSY1, MTF1, M
TO1, NAM1, NAM2, NAM9, NDI1,
NHX1, NUC1, OM45, ORF_A04514, OSM1, OXA1, PDA1, PDB1, PDX1
, PEL1, PET111, PET112, PET117, PET122, PET123, PET127, PET130,
PET191,
PET309, PET494, PET54, PET56, PET9, PETCR46, PHB1, PHB2, PIF1,
PIM1,
POR1, POR2, PPA2, PSD1, PUT1, PUT2, QCR10, QCR2, QCR6, QCR7,
QCR8, QCR9, RCA1, RF2, RIM1, RIM2, RIP1, RML2, RNA12, RPM2, RP
O41, SCO1,
SCO2, SDH1, SDH2, SDH3, SDH4, SECY, SHM1, SHY1, SLS1, SMF2,
SOD2,
SOM1, SSC1, SSQ1, STF1, STF2, SUN4, SUV3, TIM17, TIM22, TIM23
,
TIM44, TIM54, TOM20, TOM22, TOM37, TOM40, TOM6, TOM7, TOM
70,
TOM72, TRM1, TUF1, UNG1, VAR1, YAH1, YAL011W, YAT1, YBL013

W,
YCR024C, YDR041W, YDR115W, YDR116C, YER073W, YFH1, YGL06
8W,
YGR257C, YHM1, YHR075C, YHR148W, YJL200C, YJR113C, YKL055
C,
YKL120W, YKL134C, YKL192C, YLR168C, YMC1, YMC2, YML025C,
YMR188C, YMR31, YNL081C, YNL306W, YNR036C, YNR037C, YOR2
21C,
YPL013C, ETF-BETA

PEROXISOMAL

CAT2, CIT2, CTA1, DAL7, EHD1, EHD2, FAA2, FAT2, FOX2, ICL1, IDP
3,
MDH3, MLS1, PEX11, PEX12, PEX13, PEX14, PEX17, PEX2, PEX3, PE
X4,
PEX6, PEX7, PEX8, POT1, POX1, PXA1, PXA2, SPS19, YBR204C, YDR
449C, YHR180W

DNA-ASSOCIATED

A1, A2, ABF1, ABF2, ADA2, ADE12, ADR1, ALPHA1, ALPHA2, ANC1,
APN1, ARGR1, ARGR2, ARGR3, ARR1, ASH1, AZF1, BAS1, BDF1, BR
F1, BUR6,
CAC2, CAD1, CAF17, CAT8, CBF1, CBF2, CCE1, CCR4, CDC13, CDC3
6,
CDC39, CDC46, CDC47, CDC54, CDC6, CDC7, CDC73, CDC9, CEF1,
CEP3,
CHA4, CHD1, CHL1, CHL4, CRZ1, CSE1, CSE2, CSE4, CTF13, CUP2,
CUP9,
DAL80, DAL81, DAL82, DAT1, DBF4, DMC1, DNA2, DNA43, DNL4, D
OS2,
DOT6, DPB11, DPB2, DPB3, DST1, ECM22, ENS2, EST1, EZL1, FCP1,
FHL1,
FKH1, FKH2, FLO8, FZF1, GAL11, GAL4, GAT1, GBP2, GCN4, GCN5,
GCR1,
GCR2, GLN3, GLO3, GTS1, GZF3, HAC1, HAP1, HAP2, HAP3, HAP4,
HCM1,
HDA1, HDF1, HFM1, HHF1, HHF2, HHO1, HHT1, HHT2, HMO1, HMS1,
HMS2, HO, HOP1, HPR1, HPR5, HSF1, HTA1, HTA2, HTA3, HTB1, HT
B2, IFH1,

IME1, IME4, INO2, INO4, IXR1, KAR4, LEU3, LYS14, LYS20, LYS21, MAC1,
MAG1, MAL13, MAL23, MAL33, MATALPHA1, MATALPHA2, MBP1, MCD1, MCM1, MCM2, MCM3, MCM6, MED6, MER2, MET18, MET28, MET30,
MET31, MET32, MET4, MGA2, MGT1, MIF2, MIG1, MIG2, MIP1, MLH1,
MOL1, MOT1, MPT4, MRE11, MSH1, MSH2, MSH3, MSH4, MSH5, MSI1,
MSN1, MSN2, MSN4, MTF1, NBN1, NCB2, NDJ1, NGG1, NHP2, NHP6A,
NHP6B, NOT3, NUC2, OAF1, OPI1, ORC1, ORC2, ORC3, ORC4, ORC5, ORC6, PAF1, PCH1, PCH2, PDR1, PDR3, PGD1, PHD1, PHO2, PHO4, PHR1, PIF1, PIP2, PMS1, POB1, POL1, POL12, POL2, POL3, POL30, POL4, POP2, PPR1, PRI1, PRI2, PSO2, PUT3, RAD1, RAD10, RAD14, RAD16, RAD18, RAD2, RAD23,
RAD26, RAD27, RAD3, RAD4, RAD5, RAD50, RAD51, RAD52, RAD54,
RAD55, RAD57, RAD6, RAD7, RAP1, RAT1, RCS1, REB1, REC102, REC104,
REC114, RED1, REG1, RET1, REV3, RFA1, RFA2, RFA3, RFC1, RFC2, RFC3,
RFC4, RFC5, RGM1, RGT1, RIF1, RIF2, RIM1, RIM101, RLF2, RLM1, RME1, RMS1, ROX1, ROX3, RPA12, RPA135, RPA14, RPA190, RPA34, RPA43, RPA49, RPB10, RPB11, RPB2, RPB3, RPB4, RPB5, RPB6, RPB7, RPB8, RPB9, RPC10, RPC19, RPC25, RPC31, RPC34, RPC40, RPC53, RPC82, RPD3, RPO21, RPO31,
RPO41, RRN10, RRN11, RRN3, RRN5, RRN6, RRN7, RRN9, RSC4, RSC6,
RSC8, RTG1, RTG3, SAS5, SEF1, SET1, SFH1, SFL1, SGS1, SIG1, SIN3, SIN4, SIP2, SIP4, SIR1, SIR2, SIR3, SIR4, SKN7, SKO1, SMC1, SMC2, SMP1, SNF2, SNF5, SNF6, SOK2, SPK1, SPO1, SPS18, SPT10, SPT15, SPT16, SPT2,
SPT21, SPT23, SPT3, SPT4, SPT5, SPT6, SPT8, SRB2, SRB4, SRB5, SRB6,
SRB7, SRB8, SRB9, SSL2, SSN3, SSN6, SSN8, SSU72, STB4, STB5, STE12,
STH1, SUA7, SWI1, SWI3, SWI4, SWI6, SWP73, TAF19, TAF25, TBF1, TEA1, TEC1, TFA1, TFA2, TFB1, TFB2, TFB3, TFB4, TFC1, TFC2, TFC3, TFC4,
TFC5, TFG1, TFG2, THI2, TOA1, TOA2, TOP1, TOP2, TOP3, TRF4, TSP1,
TUP1, TYE7, UGA3, UME6, UNG1, USV1, XRS2, YAL019W, YAP1, YAP3,

YAP5, YBL054W, YBR026C, YBR150C, YBR239C, YCR106W, YDR026C,
YDR060W, YDR213W, YER045C, YER184C, YFL052W, YIL036W, YIL130W,
YJL103C, YJL206C, YKL005C, YKL222C, YKR064W, YLL054C, YLR087C,
YLR266C, YNL206C, YOL089C, YOR172W, YOR380W, YOX1, YPL133C,
YPR008W, YPR196W, YRR1, ZAP1, ZIP1, ZUO1

IMMUNOSUPPRESENT

FEN1, SSH4, SHR3

CYCLINS

CCL1, CLB1, CLB2, CLB3, CLB4, CLB5, CLB6, CLG1, CLN1, CLN2, CLN3,
CTK2, PCL1, PCL10, PCL2, PCL5, PCL6, PCL7, PCL8, PCL9, PHO80, SSN8,
YBR095C

ATP-BINDING CASSETTE PROTEINS

ADP1, ATM1, CAF16, GCN20, MDL1, MDL2, PDR10, PDR11, PDR12,
PDR15, PDR5, PXA1, PXA2, SNQ2, STE6, YBT1, YCF1, YDL223C, YDR091C, YEF3B, YER036C, YHL035C, YKR103W, YKR104W, YLL015W, YNR070W, YOR011W, YOR1, YPL226W

CYTOSKELETAL

ABP1, ACF2, ACT1, AFR1, AIP1, AIP2, ARP3, AUT2, AUT7, BEM1, BIM1,
BNI1, BNI4, BUD3, BUD6, CAP1, CAP2, CDC10, CDC11, CDC12, CDC3, CIN1, CIN2, CIN4, CMD1, COF1, CRN1, END3, GIC1, GIC2, GIN4, JNM1, KAR9,
KIP2, KIP3, LAS17, MDM1, MHP1, MYO1, MYO2, MYO3, MYO4, MYO5,
PFY1, RVS161, RVS167, SAC6, SAC7, SEC1, SHE3, SHM2, SLA1, SLA2,

SMY1, SMY2, SPA2, SPH1, SPR28, SPR3, SRV2, TCP1, TPM1, TPM2, TUB1, TUB2, TUB3, VPS16, VRP1

APOPTOSIS

ATP1, ATP14, ATP15, ATP16, ATP2, ATP3, ATP4, ATP5, ATP6, ATP7, ATP8, ATP9, CYC1, SHO1, SSK2, SSK22, SWI3, SXM1

ADDITIONAL CELL RESCUE

ACC1, ALD6, BCK1, BEM1, BEM2, BIM1, BMH1, BMH2, CAN1, CBF1, CDC1, CDC14, CDC15, CDC20, CDC25, CDC28, CDC33, CDC37, CDC42, CDC43,
CDC53, CDC6, CHC1, CIN8, CKA1, CKA2, CLA4, CLB1, CLB2, CLB3, CLB4,
CLB5, CLN1, CLN2, CLN3, CMP2, CNA1, COF1, CTT1, DBF2, DBF20, DPM1, ERG25, GIC1, GIC2, GPA1, GRR1, HCA4, HIS4, HOC1, HSF1, KAR1, KES1,
KRE6, KSS1, MBP1, NMT1, ORC2, ORC5, PDE2, PEP12, PEP7, PKC1, PLC1,
PMR1, POL30, PRP18, RAM1, RAS1, RAS2, RBL2, RED1, RFC1, RHO1, RHO3, RHO4, SAC1, SEC13, SEC14, SEC22, SEC4, SET1, SIS2, SKP1, SPC98, SRA1, SRO4, SRP1, SSA1, SSA2, SSA4, SSN8, STE20, STN1, STT4, SUI3, SWE1,
SWI4, SWI6, TEL1, TOR1, TUB1, TUB4, VMA1, YCK1, YCK2, YPT1

ADDITIONAL CELL DAMAGE

APN1, BUB1, CDC28, CDC45, CDC46, CDC47, CDC54, CDC7, CLB1, CLB2,
CLB3, CLB5, DDC1, DDR2, DDR48, DIN7, DUN1, ECM32, HSM3, IMP2, MEC1, MEC3, MGT1, MOL1, MRE11, MUS81, NTG1, PDS1, PGD1, PHR1, POL2,
POL3, POL30, POL4, PRI1, PSO2, RAD14, RAD16, RAD17, RAD18, RAD24,
RAD30, RAD51, RAD52, RAD54, RAD55, RAD57, RAD7, RAD9, RDH54,
REV3, RFA1, RFC5, RNR1, RNR2, RNR3, RNR4, RPH1, SIC1, SML1, SPK1,
STN1, STS1, TEL1, TFA1, TFA2, TUP1, UBC7, UBI4, XBP1, YBR098W, YFH1

OTHER RELEVANT MUTANTS AND GENES

Y-1, 9520b, C658-K7, JPD 4, JPM 9, Cy32, E354, JC488, PSY 142, OI-2, Y217, JC787-9A, ML1-21, Y500, 86-9C, GL1, GT5-1A, HD56-5A, PZ1,
127-4D, Y229, JC302-26B, JC482, LB2211-2B, MH41-7B/P21, erg 8-1, SEY6211, GL4, K335, MK20, MK34, DE4-3A, DE4-3B, DE4-3C, MMYO11, UH1-GRGZ, 2150-2-3a, Y211, DP1/517, 943, 1117, C658, 1252, H79.20.3, LB1-3B, C658-K42, R29B, LB54-3A, XW520-9A, ade7, D225-5A, 309, SDH1, SDH2, SDH3, SDH4, TCM62, PDE1, PDE2

Appendix B

NONLIMITING LIST OF MAMMALIAN GENES

11-beta hydroxysteroid dehydrogenase type II,12-lipoxygenase, 17-beta hydroxysteroid dehydrogenase, 60S ribosomal protein L6, 6-O-methylguanine-DNA methyltransferase, Activating transcription factor 2, Activating transcription factor 3, Activating transcription factor 4, Activin beta E, Activin receptor type II, Acyl - CoA dehydrogenase, Acyl CoA Carrier Protein, Adenine nucleotide translocator 1, Alanine aminotransferase, Alcohol dehydrogenase 1, Alcohol dehydrogenase 2, Alcohol dehydrogenase 3, Alcohol dehydrogenase 4, Alcohol dehydrogenase 5, Aldehyde dehydrogenase 1, Aldehyde dehydrogenase 2, Aldehyde dehydrogenase 3, Alpha 1-antitrypsin, Alpha-1 acid glycoprotein, Alpha-1 antichymotrypsin, Alpha-catenin, Alpha-tubulin, Apolipoprotein A1, Apolipoprotein AII, Apolipoprotein CIII, Apolipoprotein E, Aryl hydrocarbon receptor, Aspartate aminotransferase, mitochondrial, Ataxia telangeictasia, ATP-dependent helicase II (70kDa), ATP-dependent helicase II (Ku80), BAG-1, BAK, Bax (alpha), Bcl-2, Bcl-xL, Beta-actin, Bilirubin UDP-glucuronosyltransferase isozyme 1, Bilirubin UDP-glucuronosyltransferase isozyme 2, Biliverdin reductase, Branched chain acyl-CoA oxidase, BRCA1, BR-cadherin, C4b-binding protein, c-abl, Calcineurin-B, Calnexin, Calprotectin, Calreticulin, canalicular multispecific organic anion transporter, Carbonic Anhydrase III, Carnitine palmitoyl-CoA transferase, Caspase 1, Caspase 2 (Nedd2), Caspase 3 (CPP32-beta), Caspase 5 (ICE rel-III), Caspase 6 (Mch2-alpha), Caspase 7 (Mch3-alpha), Caspase 8 (FLICE), Catalase, Catechol-O-methyltransferase, CCAAT/enhancer-binding protein alpha, CCAAT/enhancer-binding protein epsilon, Cell division cycle protein 2, Cell division cycle protein 20, Cell division cycle protein 25, Cellular retinoic acid binding protein 1, Cellular retinoic acid binding protein 2, c-erb, c-fos, Checkpoint kinase-1, Cholesterol esterase, c-H-ras, c-jun, Clusterin, c-myc, Complement component C3, Connexin 30, Connexin-32, Connexin-40, Corticosteroid binding globulin, Corticotropin releasing factor, C-reactive protein, Creatine kinase b, Cyclin D1, Cyclin dependent kinase 1, Cyclin dependent kinase 4, Cyclin dependent kinase inhibitor 1A, Cyclin E, Cyclin G, Cyclin-dependent kinase 4 inhibitor (P16), Cyclin-dependent kinase 4 inhibitor B (P16), Cyclin-dependent kinase inhibitor P27Kip1, Cyclooxygenase 2, Cystic fibrosis transmembrane conductance regulator, Cytochrome P450 11A1, Cytochrome P450 17A, Cytochrome P450 1A1, Cytochrome P450 1A2, Cytochrome P450 1B1, Cytochrome P450 2A1, Cytochrome P450 2A3, Cytochrome P450 2A6, Cytochrome P450 2B1, Cytochrome P450 2B10, Cytochrome P450 2B2, Cytochrome P450 2C11, Cytochrome P450 2C12, Cytochrome P450 2C19, Cytochrome P450 2C9, Cytochrome P450 2D6, Cytochrome P450 2E1, Cytochrome

P450 2F2, Cytochrome P450 3A1, Cytochrome P450 3A4, Cytochrome P450 4A, Cytochrome P450 4A1, Damage-specific DNA binding protein p48 subunit, Defender against cell death-1, Deleted in colorectal cancer, Delta-like protein, Dihydrofolate reductase, Disulfide isomerase related protein (ERp72), DNA binding protein inhibitor ID2, DNA dependent helicase, DNA dependent protein kinase, DNA ligase I, DNA ligase IV, DNA mismatch repair protein (MLH1), DNA mismatch repair protein (PMS2), DNA mismatch repair/binding protein (MSH3), DNA polymerase alpha, DNA polymerase beta, DNA polymerase beta, DNA repair and recombination homologue (RAD 52), DNA repair helicase II ERCC-3, DNA repair protein (RAD 50), DNA repair protein (XRCC1), DNA repair protein XP-D, DNA replication factor C (36kDa), DNA topoisomerase I, DNA topoisomerase II, Dopamine beta-hydroxylase, DRA, Dynein light chain 1, E2F, Early growth regulated protein 1, E-Cadherin, ECE-1 (endothelin converting enzyme), Endothelin-1, Enolase alpha, Enoyl CoA hydratase, Eotaxin, Epidermal growth factor, Epoxide hydrolase, ERA-B, ERCC 1 (excision repair protein), ERCC 3 (DNA repair helicase II), ERCC 5 (excision repair protein), ERCC 6 (excision repair protein), ERK1, Erythropoietin, Erythropoietin receptor, E-Selectin, Estrogen receptor, Farnesol receptor, Fas antigen, Fas associated death domain (FADD), Fas ligand, Fas/Apo1 receptor, Fatty acid synthase, Fatty acyl-CoA oxidase, Fatty acyl-CoA synthase, FEN-1 (endonuclease), Fibrinogen gamma chain, Fibronectin receptor, FIC1, Filagrin, Flavin containing monooxygenase 1, Flavin containing monooxygenase 3, FosB, Fra-1, Fucosyl transferase (alpha-1,2-fucosyltransferase), Gadd153, Gadd45, Gamma-glutamyl hydrolase precursor, Gamma-glutamyl transpeptidase, GCLR, GCLS, Glucocorticoid receptor, Glucose-6-phosphate dehydrogenase, Glucose-regulated protein 170, Glucose-regulated protein 58, Glucose-regulated protein 78, Glucose-regulated protein 94, Glutamic-oxaloacetic transaminase, Glutaminc-pyruvic transaminase, Glutathione peroxidase, Glutathione reductase, Glutathione S-transferase alpha subunit, Glutathione S-transferase Ya, Glutathione synthetase, Glyceraldehyde 3-phosphate dehydrogenase, GOS24 (zinc finger transcriptional regulator), Granulocyte-macrophage colony-stimulating factor, Growth-arrested-specific protein 1, Growth-arrested-specific protein 3, GT mismatch binding protein, H-cadherin, Heat shock protein 12, Heat shock protein 47, Heat shock protein 70, Heat shock protein 70.1, Heat shock protein 90, Helicase-like transcription factor, Heme binding protein 23, Heme oxygenase-1, Hepatic lipase, Hepatocyte growth factor, Hepatocyte growth factor activator, Hepatocyte growth factor receptor, Hepatocyte nuclear factor 4, Histone 2A, Histone 2B, HMG CoA reductase, Hydroxyacyl CoA dehydrogenase, Hydroxysteroid sulfotransferase a, Hypoxanthine-guanine phosphoribosyltransferase, ICE-rel II (Caspase 4), ICH-2 cysteine protease = CASPASE 4, IkB-a, Insulin-like growth factor binding protein 1, Insulin-like growth factor binding protein 2, Insulin-like growth factor binding protien 3, Insulin-like growth factor I, Insulin-like growth factor II, Integrin alpha, Integrin alpha L, Integrin beta1, Integrin beta2, Intercellular adhesion molecule-1, Intercellular adhesion molecule-2, Intercellular adhesion molecule-3, Interferon

gamma, Interferon inducible protein 10, Interferon inducible protein 15, Interleukin-1 alpha, Interleukin-12, Interleukin-2, Interleukin-4, Interleukin-5, Interleukin-6, Involucrin, JNK1 stress activated protein kinase , K-cadherin, Ki67, Lactate Dehydrogenase-B, Lactoferrin, Lipopolysaccharide binding protein, Lipoprotein lipase precursor, Liver fatty acid binding protein, L-myc, Low density lipoprotein receptor, Luteinizing hormone, Lysyl oxidase, Macrophage inflammatory protein-1 alpha, Macrophage inflammatory protein-1 beta, Macrophage inflammatory protein-2 alpha, Macrophage inflammatory protein-2 beta, Macrophage inflammatory protein-3 alpha, Macrophage inflammatory protein-3 beta, Malic enzyme, MAP kinase kinase, Matrix metalloproteinase-1, Matrix metalloproteinase-2, MDM-2, MET proto-oncogene, Metallothionein 1, Metallothionein 2, Metallothionein 3, Metallothionein IA, Metallothionein IG, Metal-regulatory transcription factor-1, Mitogen activated protein kinase (P38), Mitogen inducible gene (mig-2), MOAT-B (MRP/organic anion transporter), Monoamine oxidase A, Monoamine oxidase B, Multidrug resistance-associated protein, Multidrug resistant protein-1, Multidrug resistant protein-2, Multidrug resistant protein-3 = cMOAT2, MUTL homologue (MLH1), MutS Homologue (MSH2), Myeloid cell differentiation protein-1, Na/taurocholate cotransporting polypeptide, NADPH cytochrome P450 oxidoreductase, NADPH cytochrome P450 reductase, NADPH quinone oxidoreductase-1 (DT-Diaphorase), Natural killer cell-enhancing factor B, N-cadherin, NF-kappaB (p65), Nitric oxide synthase-1, inducible , Nucleoside diphosphate kinase beta isoform, O-6-alkylguanine-DNA-alkyltransferase, OB-cadherin 1, OB-cadherin 2, Octamer binding protein 1, Octamer binding protein 2, Octamer binding protein 3, Oncostatin M, Organic anion transporter 1, Organic anion transporter 3, Organic anion transporter K1, Organic anion transporting polypeptide 1, Organic cation transporter 1, Organic cation transporter 2, Organic cation transporter 3, Organic cation transporter N1, Organic cation transporter N2, Ornithine decarboxylase, Osteopontin, Oxygen regulated protein 150, p53, PAPS synthetase, P-cadherin, PEG3 (progression elevated gene 3), Peroxisomal 3-ketoacyl-CoA thiolase 1, Peroxisomal 3-ketoacyl-CoA thiolase 2, Peroxisomal acyl-CoA oxidase, Peroxisomal fatty acyl-CoA oxidase, Peroxisome assembly factor 1, Peroxisome assembly factor 2, Peroxisome biogenesis disorder protein-1, Peroxisome biogenesis disorder protein-11, Peroxisome biogenesis disorder protein-4, Peroxisome hydratase, Peroxisome proliferator activated receptor alpha, Peroxisome proliferator activated receptor gamma, Phenol sulfotransferase, Phosphoenolpyruvate carboxykinase, Phosphoglyceride kinase, Phospholipase A2, Plasminogen activator inhibitor 2, Platelet derived growth factor B, Platelet/endothelial cell adhesion molecule-1, Poly(ADP-ribose) polymerase, Proliferating cell nuclear antigen gene, Prostaglandin H synthase, Protein kinase C beta1, Protein-tyrosine phosphatase, Putative protein tyrosine phosphatase, RAD, RAD 51 homologue, RANTES, Ref-1, Replication factor C, 40-kDa subunit (A1), Replication protein A (70 kDa subunit), Retinoblastoma, Retinoblastoma related protein (P107), Retinoid X receptor alpha, Retinoid X receptor beta, Retinoid X receptor

gamma, Ribonucleotide reductase M1 subunit, Ribosomal protein L13A, Ribosomal protein S9, RNA-dependent helicase, ROAT1 (renal organic anion transporter), Serum amyloid A1, Serum amyloid A2-alpha, Sister of p-glycoprotein, Sodium/bile acid cotransporter, Sonic hedgehog gene, SQM1, Superoxide Dismutase Cu/Zn, Superoxide dismutase Mn, T-cell cyclophilin, Tenascin, Thiopurine methyltransferase, Thioredoxin, Thrombospondin 2, Thymidine kinase, Thymidylate synthase, Thymosin beta-10, Tissue inhibitor of metalloproteinases-1, Tissue transglutaminase, Transcription factor IID, Transferrin, Transforming growth factor-beta 3, Tumor necrosis factor associated factor 2 (TRAF2), Tumor necrosis factor receptor 1, Tumor necrosis factor receptor 2, Tumor necrosis factor receptor-1 associated protein (TRADD), Tumor necrosis factor-alpha, Tumor necrosis factor-beta, Type 1 interstitial collagenase, Tyrosine aminotransferase, Tyrosine protein kinase receptor (UFO), Ubiquitin , Ubiquitin conjugating enzyme (Rad 6 homologue), Ubiquitin-homology domain protein PIC1, UDP-glucuronosyltransferase 1, UDP-glucuronosyltransferase 1A6, UDP-glucuronosyltransferase 2, UDP-glucuronosyltransferase 2B, Uncoupling protein 1, Uncoupling protein 2, Uncoupling protien 3, Urate oxidase, UV excision repair protein RAD 23 (XP-C), Vascular cell adhesion molecule 1 (VCAM-1), Vascular endothelial growth factor, Vascular endothelial growth factor D, Very long-chain acyl-CoA dehydrogenase, Vimentin, Vitellogenin, Waf1, XRCC1 (DNA repair protein).

We claim:

1. A method of determining the toxicity of a sample, the method comprising the steps of

a) exposing a wildtype and one or more mutant organisms to the sample, wherein the organism is selected from the group consisting of bacteria, yeast, and mammalian cell lines;

b) measuring the growth of the organism in the presence of the sample;

c) comparing the growth measured in step b) to the growth of the wildtype and mutant organisms not in the presence of the sample to determine the sensitivity of the organisms to the sample;

d) determining a potential toxicity of the sample where the sensitivity of the wildtype in the presence of the sample as compared to the sensitivity of the mutant in the presence of the sample is statistically significant.

2. The method of claim 1 wherein the mutants have altered gene function in a category selected from the group consisting of acute phase stress, cell adhesion, AH-response, anti-apoptosis and apoptosis, antimetabolism, anti-proliferation, arachidonic acid release, ATP depletion, cell cycle disruption, cell matrix disruption, cell migration, cell proliferation, cell regeneration, cell-cell communication, cholestasis, differentiation, DNA damage, DNA replication, early response genes, endoplasmic reticulum stress, estogenicity, fatty liver, fibrosis, general cell stress, glucose deprivation, growth arrest, heat shock, hepatotoxicity, hypercholesterolemia, hypoxia, immunotox, inflammation, invasion, ion transport, liver regeneration, cell migration, mitochondrial function, mitogenesis, multidrug resistance, nephrotoxicity, oxidative stress, peroxisome damage, recombination, ribotoxic or ribotoxic stress, sclerosis, steatosis, teratogenesis, transformation, disrupted translation, transport, and tumor suppression.

3. The method of claim 1 wherein the organism is yeast.

4. The method of claim 3 wherein the yeast used is *Saccharomyces*.

5. The method of claim 3 wherein the mutants have an altered gene function in a category selected from the group consisting of cell rescue, defense, cell death and aging, cell growth, cell division and DNA synthesis, heat shock proteins, mitochondrial, peroxisomal, DNA-associated, immunosuppressent, cyclins and cell cycle control, ATP-binding cassette proteins, cytoskeletal, and apoptosis.

6. The method of claim 5 wherein the altered gene function is in the category of DNA-associated functions.

7. The method of claim 6 wherein the mutations are one or more of Rad 6, Rad 18, Rad 51, and Rad 52.

8. The method of claim 1 wherein the organism is bacteria.

9. The method of claim 8 wherein the bacteria is *Escherichia*.

10. The method of claim 8 wherein the mutants have an altered gene function in DNA associated functions.

11. The method of claim 10 wherein the mutation is RecA.

12. The method of claim 1 wherein the organism is a mammalian cell line.

13. The method of claim 12 wherein the mutant cell line has an altered gene function in the category of DNA-associated functions.

14. The method of claim 13 wherein the mutations are one or more of HHR6B, HHR51, and HHR52.

15. The method of claim 1 wherein the measuring step comprises the step of determining the turbidity of the organism culture.

16. The method of claim 1 wherein the measuring step is selected from the group consisting of measuring incorporation of 3H-thymidine or carbon 14, a

MTT assay, a XTT assay, calcein AM, Trypan blue, neutral red uptake and colony formation assay.

17. A method of determining the toxicity of a sample, the method comprising the steps of

a) exposing a wildtype and one or more mutant yeast to the sample;

b) measuring the growth of the yeast in the presence of the sample using turbidity or light scattering;

c) comparing the growth measured in step b) to the growth of the wildtype and mutant yeast not in the presence of the sample to determine the sensitivity of the yeast to the sample;

d) determining a potential toxicity of the sample where the sensitivity of the wildtype in the presence of the sample as compared to the sensitivity of the mutant in the presence of the sample is statistically significant.

18. The method of claim 1 wherein step a) is preceded by a metabolic activation step comprising the step of incubation of the sample with a preparation comprising P450 enzymes.

19. The method of claim 18 wherein the preparation is S-9 fraction derived from liver.

20. The method of claim 18 wherein the preparation is microsomes derived from yeast or liver.

Dosed/Undosed Turbidity Test Compound: Acetyl-salicyclic acid (aspirin)/EtOH Negative Control						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
6.40×10^{-9}	97.41	97.42	100.34	99.56	99.13	99.54
3.20×10^{-8}	99.23	96.75	100.65	98.31	98.35	99.76
1.60×10^{-7}	100.06	105.61	100.96	99.10	97.77	98.16
8.00×10^{-7}	97.74	98.75	98.78	104.36	99.46	100.34
4.00×10^{-6}	94.52	114.80	100.73	99.82	101.47	100.05
2.00×10^{-5}	91.72	95.54	100.49	97.33	98.74	99.76

Figure 1A

Relative Sensitivity Test Compound: Acetyl-salicyclic acid (aspirin)/EtOH Negative Control				
Test Compound (g/ml)	Rad 6/WT Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
6.40×10^{-9}	0.0	-3.0	0.4	0.0
3.20×10^{-8}	2.5	-1.4	0.0	-1.5
1.60×10^{-7}	-5.6	-0.9	1.3	1.0
8.00×10^{-7}	-1.0	-1.1	4.7	3.9
4.00×10^{-6}	-21.4	-6.6	-1.7	-0.2
2.00×10^{-5}	-4.2	-9.6	-1.5	-2.5

Figure 1B

Dosed/Undosed Turbidity Test Compound: Cimetidine (Tagamet)/EtOH Negative Control						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
3.20×10^{-9}	96.75	96.20	99.48	100.09	99.39	98.01
1.60×10^{-8}	99.48	97.20	100.42	99.43	100.30	97.06
8.00×10^{-8}	99.31	95.87	100.26	99.10	98.81	100.56
4.00×10^{-7}	100.72	98.19	101.12	100.94	100.56	99.17
2.00×10^{-6}	99.31	98.30	100.73	101.93	101.67	100.56
1.00×10^{-5}	101.62	100.96	101.27	100.22	101.47	100.05

Figure 2A

Relative Sensitivity Test Compound: Cimetidine (Tagamet)/EtOH Negative Control				
Test Compound (g/ml)	Rad 6/WT Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
3.20×10^{-9}	0.6	-2.8	0.7	2.1
1.60×10^{-8}	2.3	-0.9	-0.9	2.4
8.00×10^{-8}	3.5	-1.0	0.3	-1.5
4.00×10^{-7}	2.5	-0.4	0.4	1.8
2.00×10^{-6}	1.0	-1.4	0.3	1.3
1.00×10^{-5}	0.7	0.3	-1.3	0.2

Figure 2B

Dosed/Undosed Turbidity Test Compound: Oligomycin (Antibiotic)/EtOH Negative Control						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
6.40×10^{-9}	97.83	97.75	100.03	99.17	98.94	98.96
3.20×10^{-8}	97.83	97.31	100.73	98.77	99.00	96.70
1.60×10^{-7}	97.74	101.18	101.59	93.45	94.72	98.52
8.00×10^{-7}	102.45	105.50	102.91	95.88	97.64	101.07
4.00×10^{-6}	99.15	103.73	97.77	93.64	93.03	95.02

Figure 3A

Relative Sensitivity Test Compound: Oligomycin (Antibiotic)/EtOH Negative Control				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
6.40×10^{-9}	0.1	-2.2	0.2	0.2
3.20×10^{-8}	0.5	-3.0	-0.2	2.1
1.60×10^{-7}	-3.5	-3.9	-1.4	-5.4
8.00×10^{-7}	-3.0	-0.5	-1.8	-5.4
4.00×10^{-6}	-4.6	1.4	0.7	-1.5

Figure 3B

Dosed/Undosed Turbidity Test Compound: Actinomycin D (DNA Binder)/Media Positive Test						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
3.20×10^{-10}	108.3	97.9	102.9	94.7	97.4	95.7
1.60×10^{-9}	110.5	99.8	100.6	94.5	97.6	94.3
8.00×10^{-9}	110.2	95.1	98.3	94.6	97.2	93.7
4.00×10^{-8}	109.4	84.6	93.1	94.5	95.8	93.5
2.00×10^{-7}	99.7	65.5	82.4	94.7	94.8	93.3
1.00×10^{-6}	79.6	55.1	76.3	92.9	87.9	88.1

Figure 4A

Relative Sensitivity Test Compound: Actinomycin D (DNA Binder)/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
3.20×10^{-10}	9.6	5.0	-2.8	-1.1
1.60×10^{-9}	9.7	8.9	-3.2	0.3
8.00×10^{-9}			-2.8	1.0
4.00×10^{-8}			-1.4	1.0
2.00×10^{-7}			-0.2	1.4
1.00×10^{-6}		4.2	5.4	5.2

Figure 4B

Dosed/Undosed Turbidity Test Compound: Carboplatin (Antineoplastic Agent)/Media Positive Test						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
4.61×10^{-8}	103.8	96.1	102.3	95.7	97.0	96.5
2.30×10^{-7}	108.2	99.7	99.4	94.7	96.0	93.7
1.15×10^{-6}	107.3	94.0	93.6	93.4	95.9	91.9
5.76×10^{-6}	107.7	81.7	80.3	94.5	94.1	91.3
2.88×10^{-5}	94.3	62.7	64.5	95.8	89.1	86.1
1.44×10^{-4}	82.1	54.2	55.4	92.9	83.1	81.9

Figure 5A

Relative Seasitivity Test Compound: Carboplatin (Antineoplastic Agent)/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
4.61×10^{-8}	7.4	1.5	-1.4	-0.8
2.30×10^{-7}	7.8	8.1	-1.4	1.0
1.15×10^{-6}			-2.7	1.6
5.76×10^{-6}			0.5	3.4
2.88×10^{-5}			7.0	
1.44×10^{-4}				

Figure 5B

Dosed/Undosed Turbidity Test Compound: EMS (Alkylating Agent)/Media Positive Test						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
3.20×10^{-8}	103.8	96.8	100.9	95.9	95.3	95.6
1.60×10^{-7}	109.1	98.3	97.0	94.5	96.2	93.3
8.00×10^{-7}	106.8	91.6	83.5	95.2	95.9	90.6
4.00×10^{-6}	102.0	74.8	65.9	95.1	91.2	86.2
2.00×10^{-5}	81.8	57.7	52.2	93.8	77.6	72.4
1.00×10^{-4}	70.0	48.5	44.3	86.7	64.1	57.0

Figure 6A

Relative Sensitivity Test Compound: EMS (Alkylating Agent)/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
3.20×10^{-8}	6.7	2.8	0.6	0.3
1.60×10^{-7}	9.9	[illegible]	-1.8	1.3
8.00×10^{-7}	[illegible]	[illegible]	-0.8	4.8
4.00×10^{-6}	[illegible]	[illegible]	4.1	9.3
2.00×10^{-5}	[illegible]	[illegible]	[illegible]	[illegible]
1.00×10^{-4}	[illegible]	[illegible]	[illegible]	[illegible]

Figure 6B

Dosed/Undosed Turbidity Test Compound: 5-FU (Antimetabolite)/Media Positive Test						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
1.28×10^{-8}	96.61	94.32	99.80	98.26	99.23	99.58
6.40×10^{-8}	94.98	96.74	99.72	99.08	99.91	101.19
3.20×10^{-7}	97.04	98.52	99.12	102.45	99.91	101.47
1.60×10^{-6}	95.85	91.78	87.42	101.35	90.45	99.79
8.00×10^{-6}	79.93	64.45	63.77	83.88	63.97	73.86
4.00×10^{-5}	50.36	40.30	37.55	56.42	33.14	39.58

Figure 7A

Relative Sensitivity Test Compound: 5-FU (Antimetabolite)/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
1.28×10^{-8}	2.4	-3.3	-1.0	-1.3
6.40×10^{-8}	-1.8	-5.0	-0.8	-2.1
3.20×10^{-7}	-1.5	-2.1	2.5	1.0
1.60×10^{-6}	4.2	8.8	[illegible]	1.5
8.00×10^{-6}	[illegible]			
4.00×10^{-5}	[illegible]			

Figure 7B

Dosed/Undosed Turbidity Test Compound: MMS (Alkylating Agent)/Media Positive Test						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
2.08×10^{-5}	98.5	64.9	50.7	94.8	79.6	76.5
1.04×10^{-4}	80.3	54.3	45.2	89.6	57.2	45.9
5.20×10^{-4}	70.9	47.1	33.4	67.5	45.1	38.5
2.60×10^{-3}	57.1	40.5	27.0	27.5	30.0	28.2
1.30×10^{-2}	41.4	29.9	21.6	17.4	20.2	21.3

Figure 8A

Relative Sensitivity Test Compound: MMS (Alkylating Agent)/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
4.16×10^{-6}	[illegible]	[illegible]	4.2	5.9
2.08×10^{-5}	[illegible]	[illegible]	[illegible]	[illegible]
1.04×10^{-4}	[illegible]	[illegible]	[illegible]	[illegible]
5.20×10^{-4}	[illegible]	[illegible]	[illegible]	[illegible]
2.60×10^{-3}	[illegible]	[illegible]	-8.8	-2.5
1.30×10^{-2}	[illegible]	[illegible]	-16.5	-22.9

Figure 8B

Dosed/Undosed Turbidity Test Compound: PMA/Media Negative Control						
Test Compound (g/ml)	Wt Y433	Rad 6	Rad 18	Wt RSY12	Rad 51	Rad 52
4.27×10^{-9}	100.5	90.8	84.0	93.4	95.1	89.6
2.13×10^{-8}	94.4	78.2	62.4	92.7	87.7	80.4
1.07×10^{-7}	81.9	59.1	51.1	90.2	71.0	62.2
5.33×10^{-7}	81.0	48.9	38.3	80.0	52.3	42.9

Figure 9A

Relative Sensitivity Test Compound: PMA/Media Positive Test				
Test Compound (g/ml)	Rad 6/Wt Y433	Rad 18/Wt Y433	Rad 51/Wt RSY12	Rad 52/Wt RSY12
1.71×10^{-10}			-2.1	-1.8
8.53×10^{-10}			-5.3	-2.1
4.27×10^{-9}	9.7		-1.8	4.0
2.13×10^{-8}			5.4	
1.07×10^{-7}				
5.33×10^{-7}				

Figure 9B

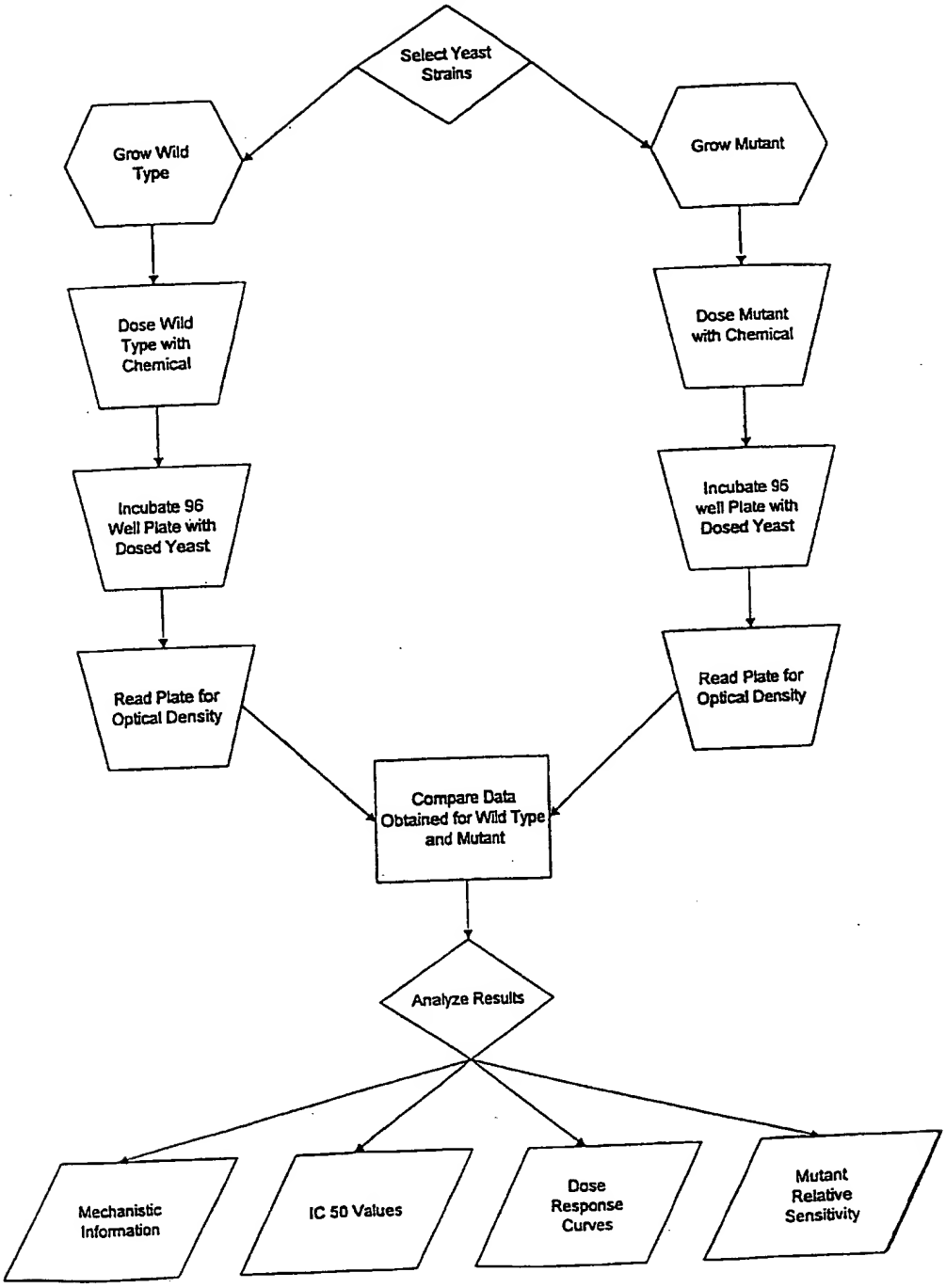
Select Yeast Strains
Grow Wild Type
Grow Mutant
Dose Wild Type with Chemical
Dose Mutant with Chemical
Incubate 96 Well Plate with Dosed Yeast
Incubate 96 well Plate with Dosed Yeast
Read Plate for Optical Density
Read Plate for Optical Density
Compare Data Obtained for Wild Type and Mutant
Analyze Results
Mechanistic Information
IC 50 Values
Dose Response Curves
Mutant Relative Sensitivity

Figure 10

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: HIGH-THROUGHPUT TOXICOLOGICAL TESTING USING CULTURED ORGANISMS AND CELLS

(57) Abstract: Methods and kits for measuring mutant hypersensitivity assay using high-throughput screening methodology to evaluate the mechanisms of toxicity of chemicals. The assay is performed in multi-well plates, such as those having 96 wells, making the process conducive to testing many compounds in a short period of time. The assay is versatile in that it can test compounds for ability to cause, for example, DNA damage, ability to mutate genetic material (mutagenicity), the ability to cause cancer (carcenogenicity), cause protein or membrane damage, energy depletion, mitochondrial damage, as well as the more general genotoxicity. Thus, the term toxicity, as used in this disclosure, is intended to encompass all of these types of effects. Furthermore, the assay can detect oxidative stress, protein damage, cell cycle disruption, energy charge and depletion, microtubule disruption or onset of metabolic competency through overexpression of human gene inserts encoding metabolism genes or incorporation of S9 fraction. In a preferred embodiment of the present invention, wildtype (wt) yeast and respective mutants are dosed with the desired chemical and yeast growth is determined using turbidimetry. Dose response curves are generated and mutant sensitivity to the compound relative to its parent (relative sensitivity) calculated. Relative sensitivities which are statistically significant indicate a hypersensitivity of the mutant to the test compound. The assay therefore provides an inexpensive, reliable, short term toxicity test which is an excellent alternative to animal testing and which provides valuable information about the mechanism of action of a compound. The present invention has applications to the pharmaceutical industry, environmental testing and clinical studies.

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B. FIELDS SEARCHED

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IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
20 April 2000	28 AUG 2000

Name and mailing address of the ISA	Authorized officer
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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1

ANHANG
Zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr.

ANNEX
To the International Search Report to the international Patent Application No.

ANNEXE
Au rapport de recherche international relativ à la demande de brevet international n°

PCT/US 00/03557 SAE 270954

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